

FIG. 1A

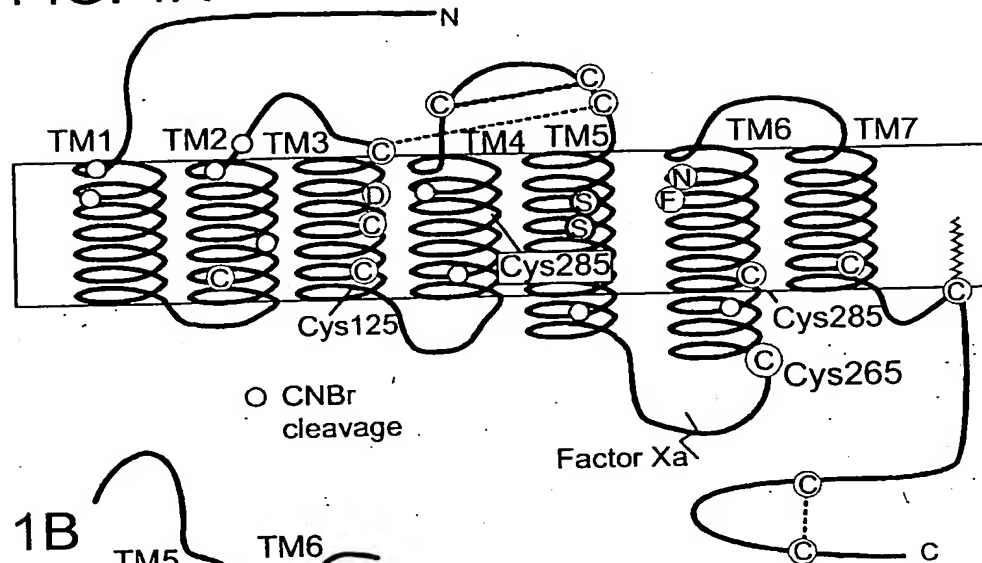
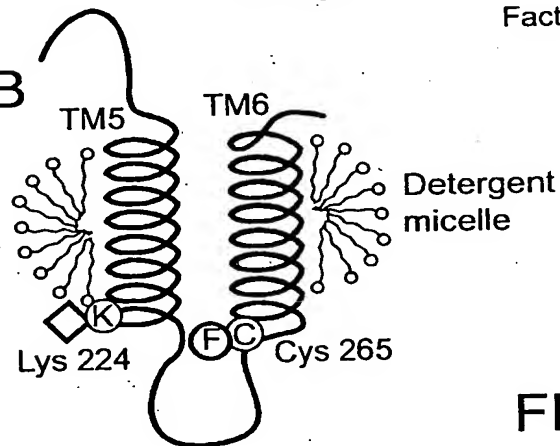
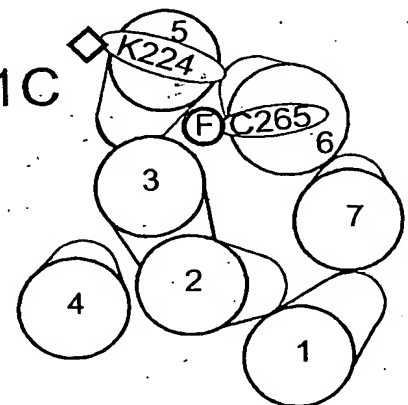


FIG. 1B



ⓕ FLUORESCHEIN
MALEIMIDE
◇ OXYL-NHS
(quencher)

FIG. 1C



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FIG. 2A

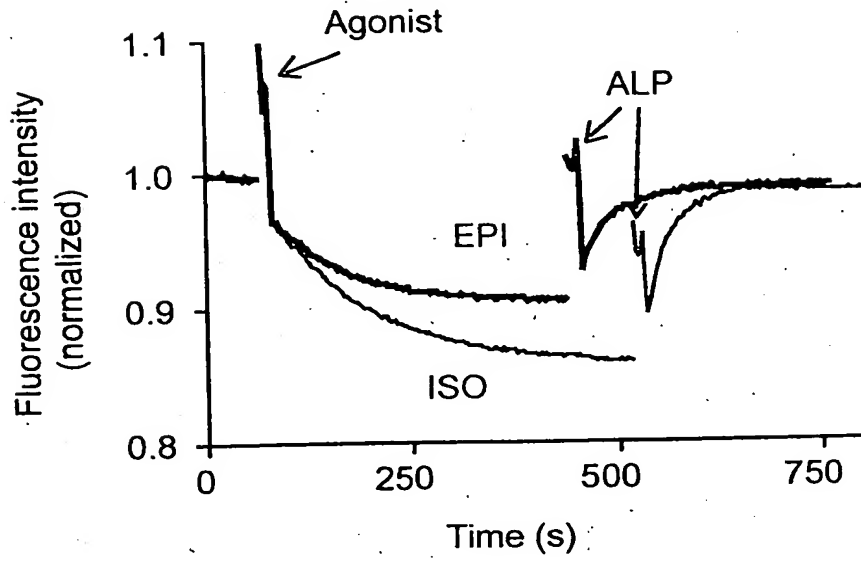
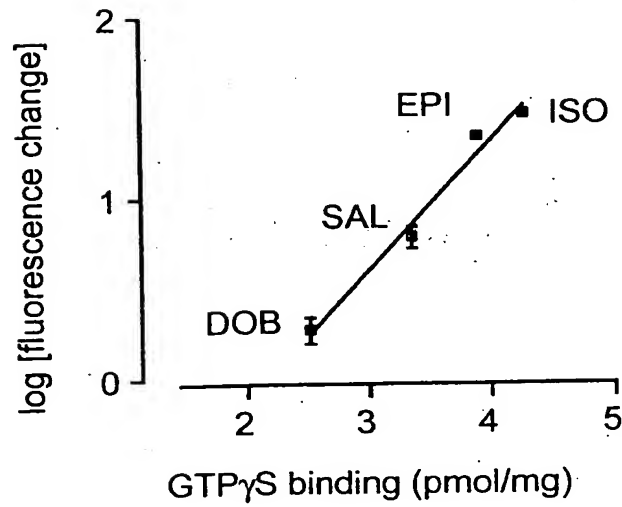


FIG. 2B



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FIG. 3A

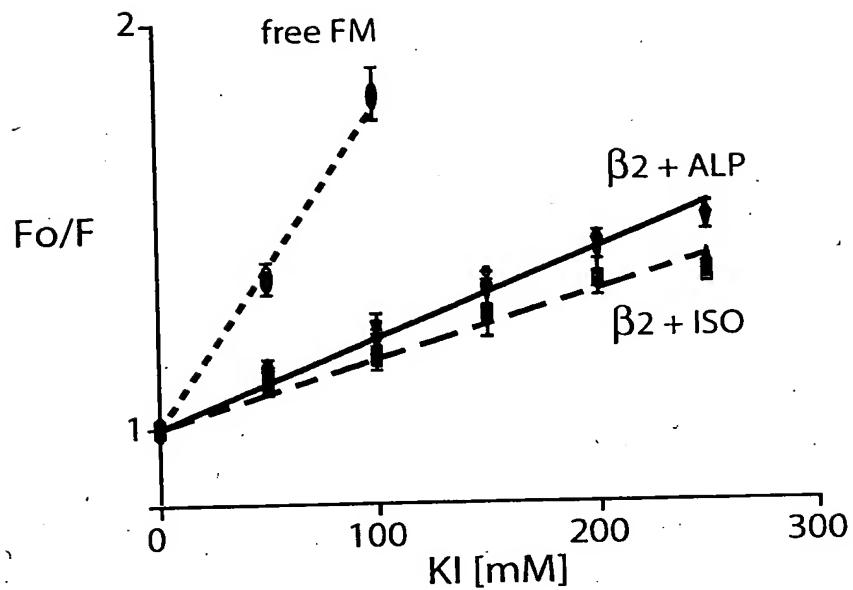
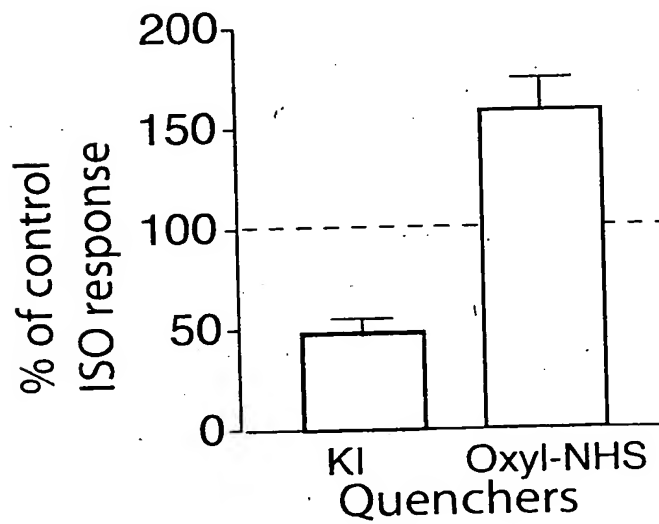


FIG. 3B



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FIG. 4A

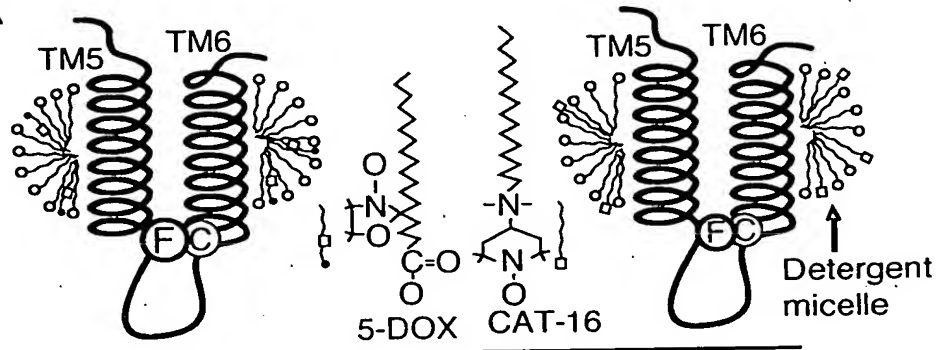


FIG. 4B

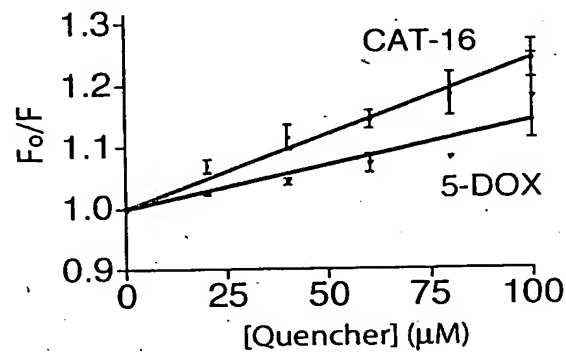


FIG. 4C

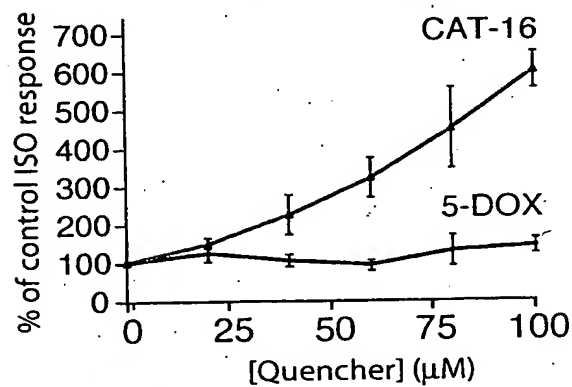
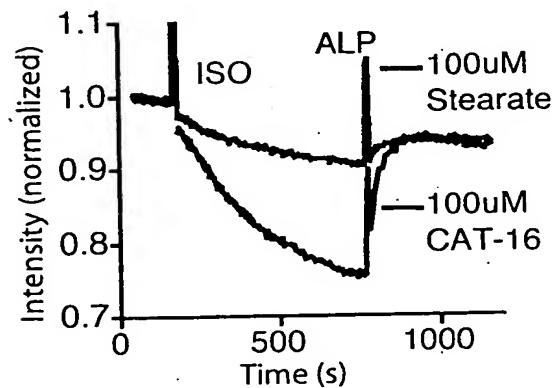


FIG. 4D



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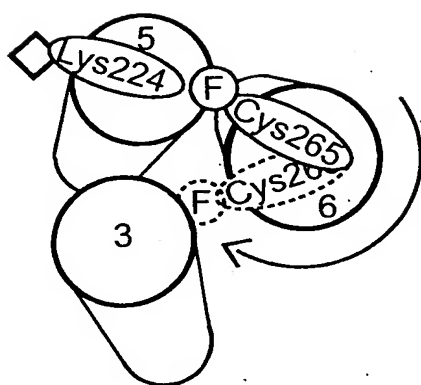


FIG. 5A

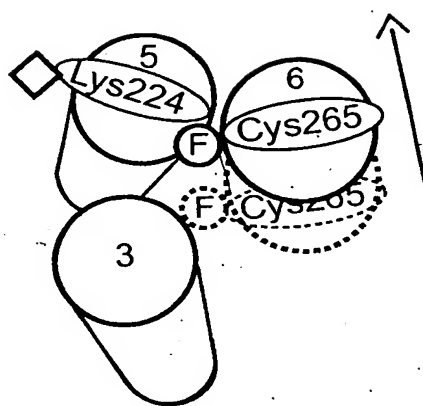


FIG. 5B

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FIG. 6A

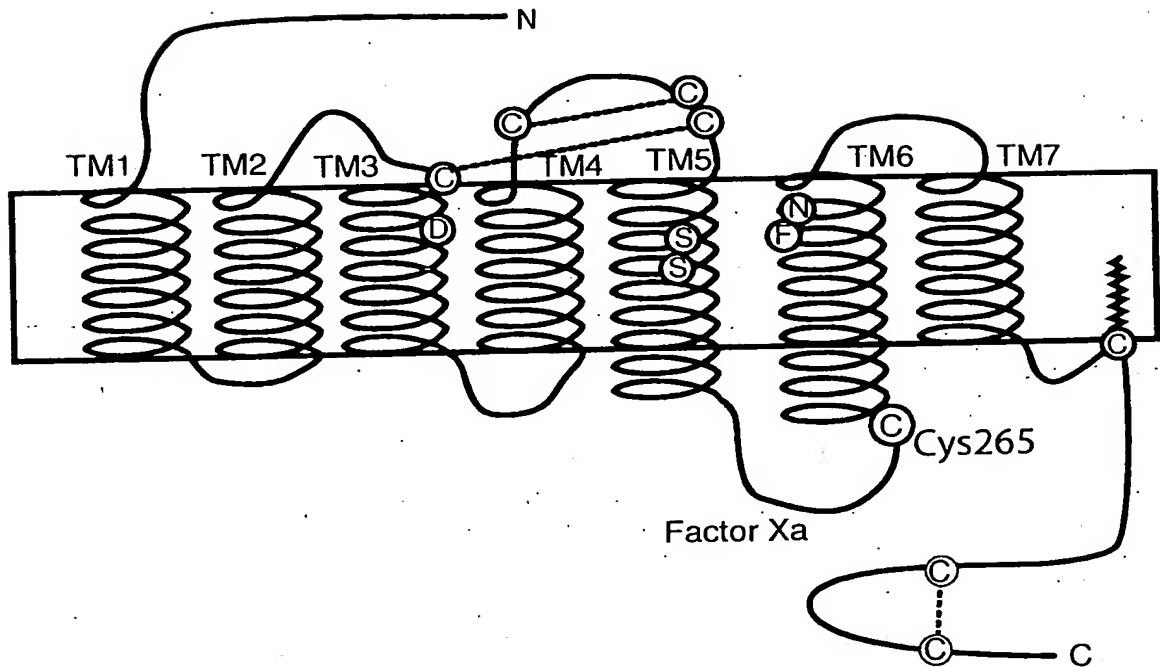
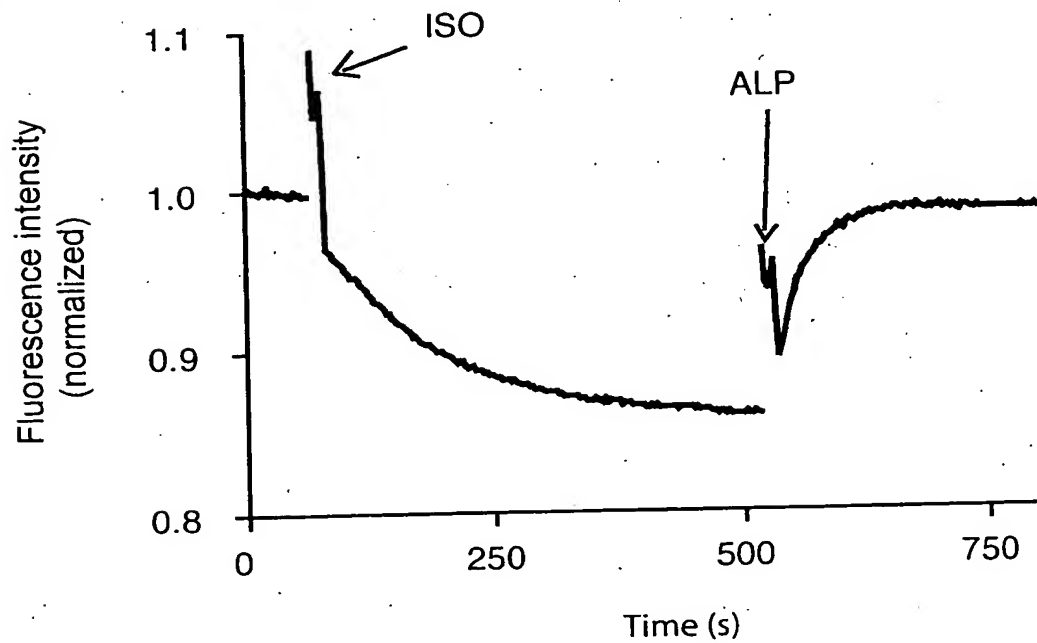
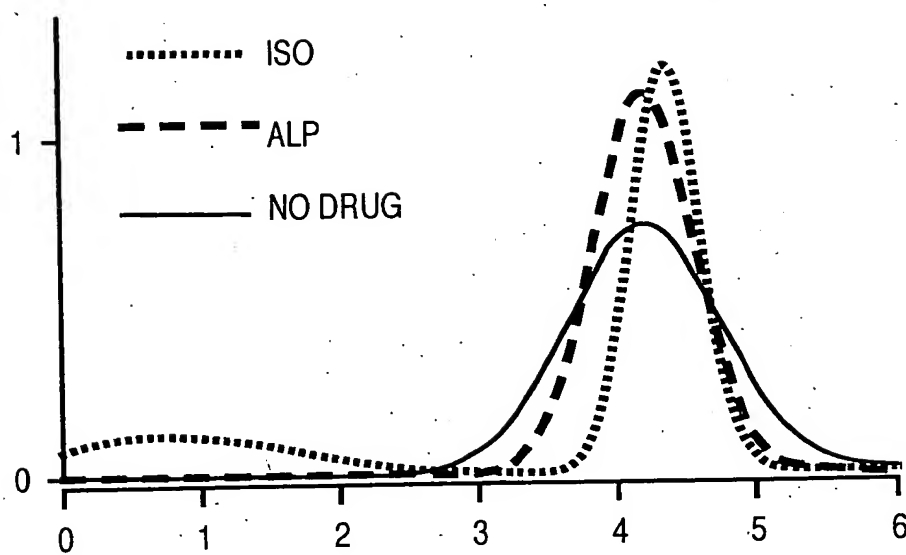


FIG. 6B



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FIG. 7



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FIG. 8A

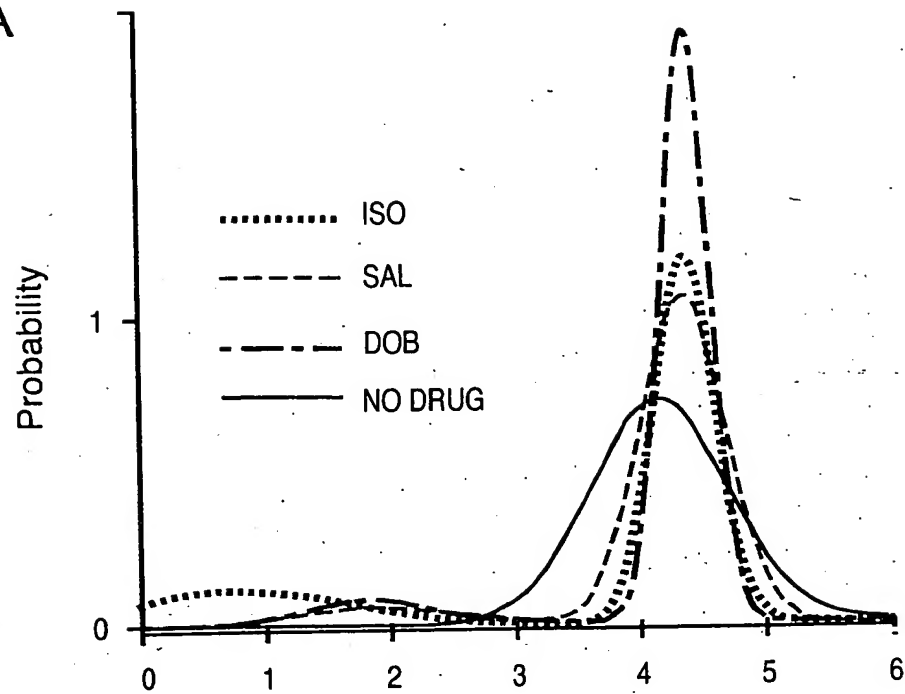
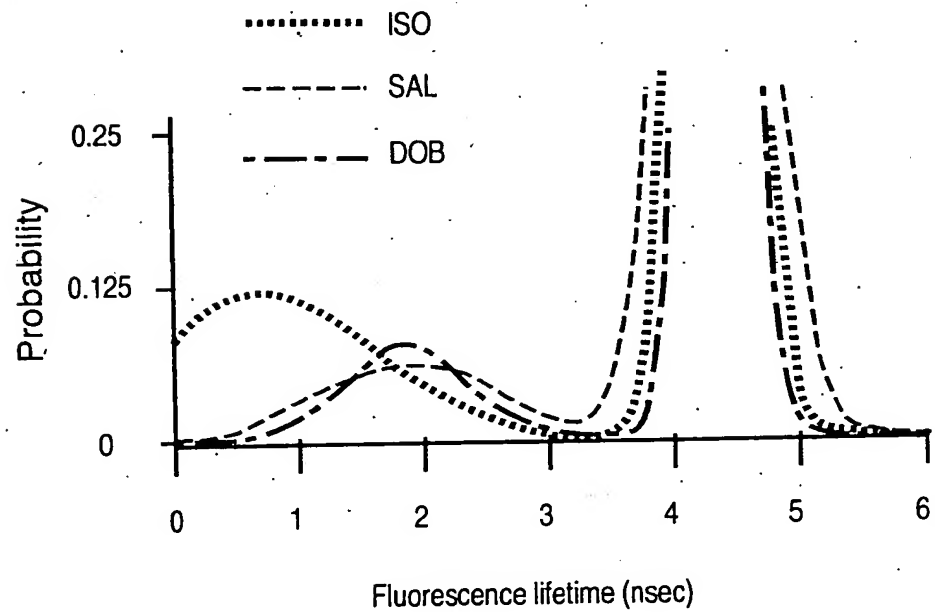


FIG. 8B



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FIG. 9A

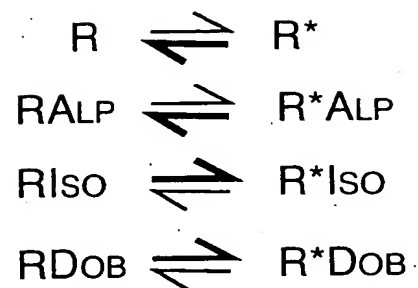
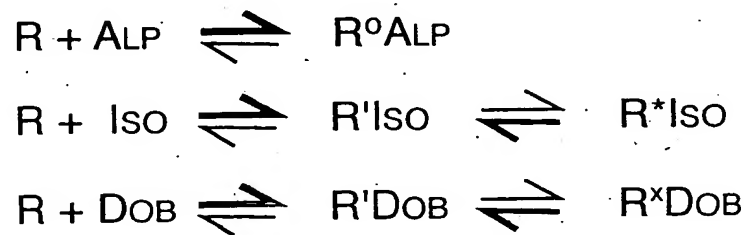
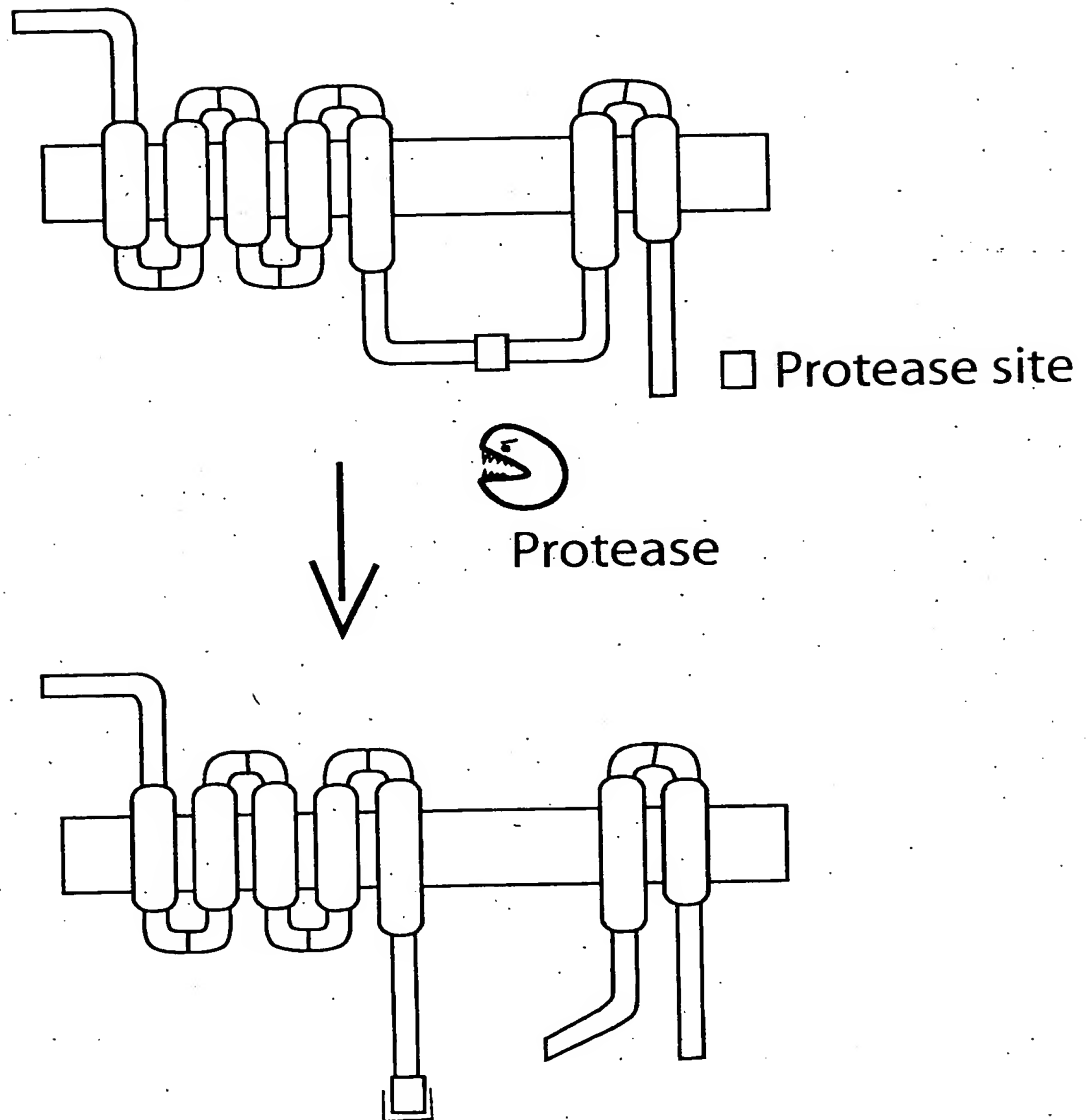


FIG. 9B

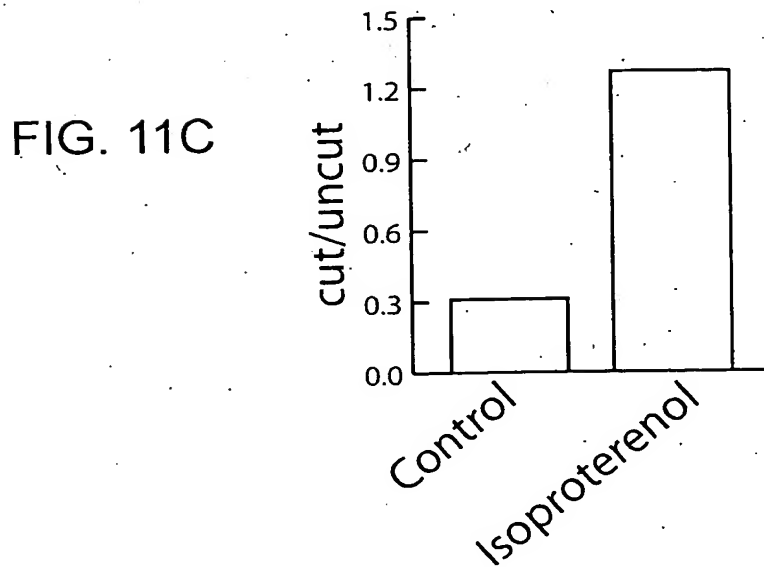
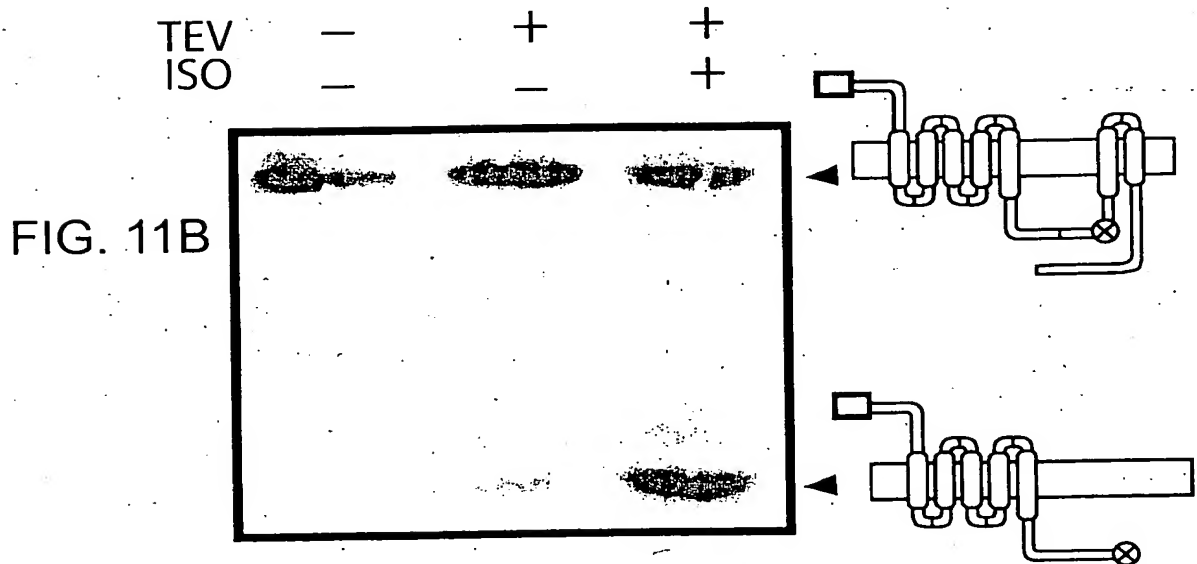
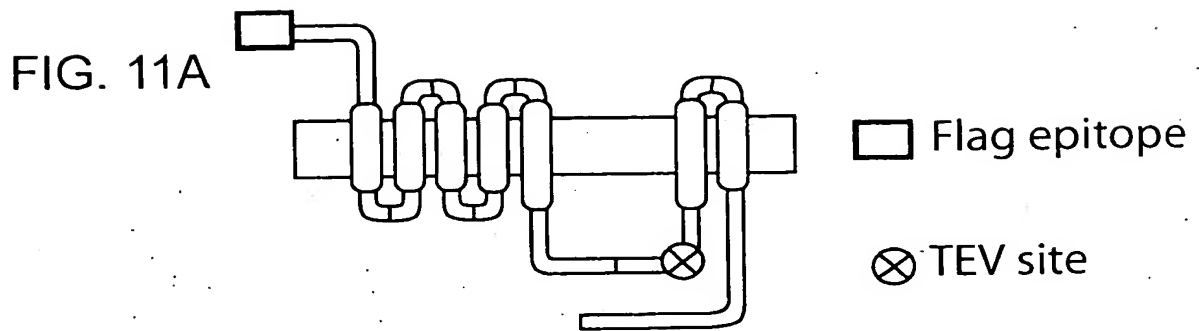


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FIG. 10



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FIG. 13
 β_2 Adrenergic Receptor DNA and Protein sequence

ATG GGG CAA CCC GGG AAC GGC AGC GCC TTC TTG CTG GCA CCC AAT AGA AGC CAT GCG CCG GAC
CAC GAC GTC CAG CAA AGG GGC GAG GTG TGG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG
ATC GTC CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG
CTG GCA GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG
TGC GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG
ATC GCA GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG
AAT AAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG
ATT CAG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG
TGC TGT GAC GAC GAC GAC GAC GAC GAC GAC GAC GAC GAC GAC GAC GAC GAC GAC GAC GAC
CCC CTG GTG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG
AAG ATT GAC GAC GAC GAC GAC GAC GAC GAC GAC GAC GAC GAC GAC GAC GAC GAC GAC GAC
CGG ACG GGC CAT GGC CAT GGC CAT GGC CAT GGC CAT GGC CAT GGC CAT GGC CAT GGC CAT
ACG TTA GGC ATC ATC ATC ATC ATC ATC ATC ATC ATC ATC ATC ATC ATC ATC ATC ATC ATC
GTG CAT GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG
TAT GTC AAT TCT TCT TCT TCT TCT TCT TCT TCT TCT TCT TCT TCT TCT TCT TCT TCT TCT
CAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG
GGC AAC ACA GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC
GAA GAC CTC CCA GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC
GAT TCA CAA GGC AGG AAT TGT AGT ACA AAT N T S C R G

FIG. 14
 β_2 Adrenergic Receptor with TEV site in 2nd intracellular loop

[illegible]

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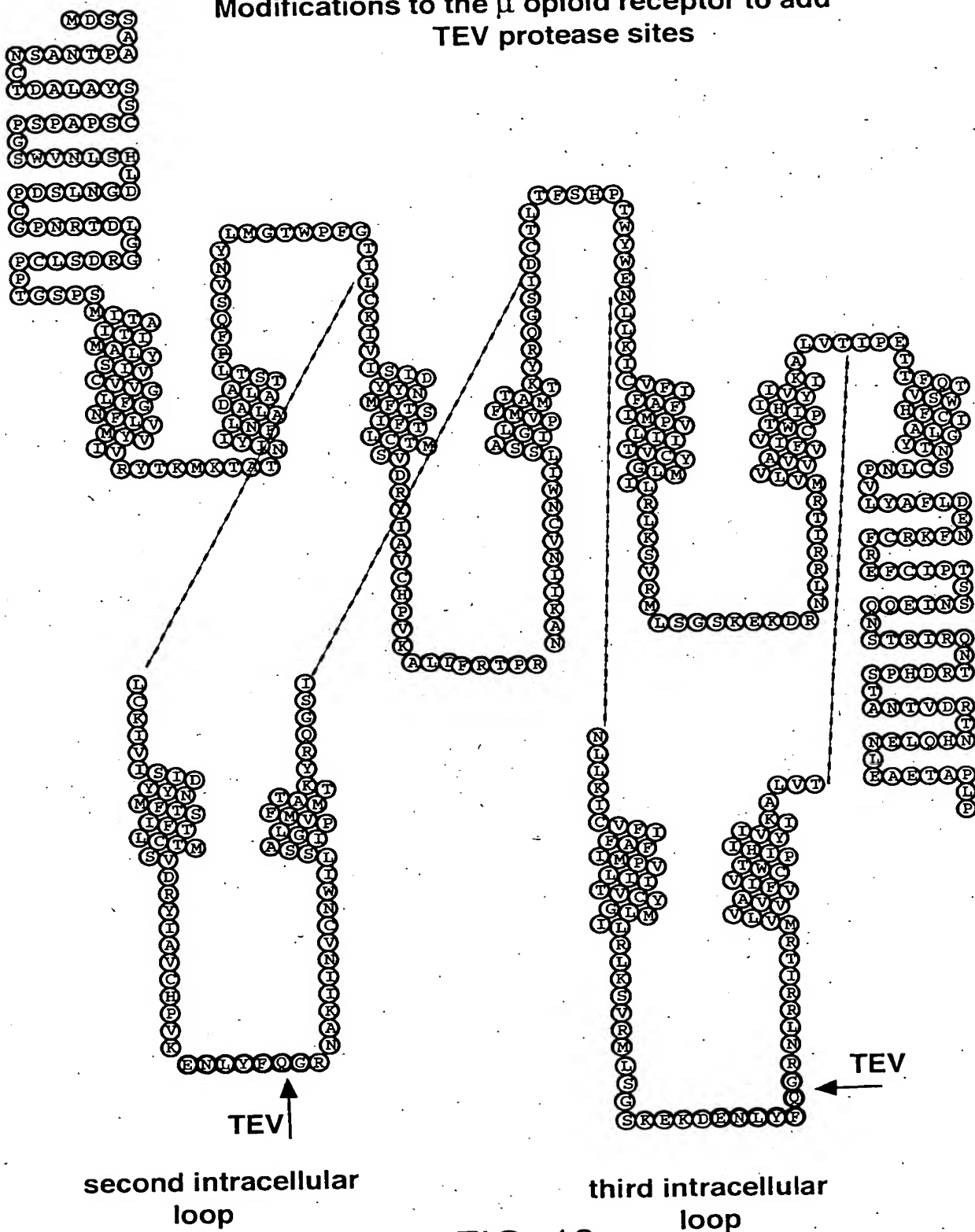
FIG. 15

b₂ Adrenergic Receptor with TEV site in 3rd intracellular loop

ATG GGG CAA CCC GGG AAC GGC AGC GCC TTC TTG CTG GCA CCC AAT AGA AGC CAT GCG CCG GAC
M G G CAG GAG CAA AGG GAC GAG GAG GTG TGG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG
H D V V Q Q R TTT GGC AAT GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG
I V L A ACC AAC TAC TTT GGC AAT GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG
L Q T T TTT GGG GGC GGC CAT ATT CTT ATG AAA ATG TGG ACT TTT GGC AAT GTG GTG GTG GTG GTG GTG
GCA GTG GTG CCC TTT GGG GGC GGC CAT ATT CTT ATG AAA ATG TGG ACT TTT GGC AAT GTG GTG GTG GTG
A V V V P P F F A A H I L
TGC GAG TTT TGG ACT TCC ATT GAT GTG CTG CTG TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC
C E F F T T TAC TTT GGC ATT ACT TCA CCT TTC AAG TAC CAG CTT TCA GGC GGC GGC GGC GGC GGC GGC
ATC GCA GTG GAT CGC TAC TTT GGC ATT ACT TCA CCT TTC AAG TAC CAG CTT TCA GGC GGC GGC GGC GGC
I A V V R Y I I T
N K A A C C T
ATT CAG ATG CAC TGG TAC CGG GGC ACC CAC CAG GAA GGC ATC ATC TCC TCT TCC TCC TCC TCC TCC TCC
I O M M H H A A C
TGT GAC TTC TTT ACG AAC CAA GCC TAT GGC ATT GGC ATT GGC ATT GGC ATT GGC ATT GGC ATT GGC
C
P L V V I I K K L
AAG ATT GAC AAA TCT GAG GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC
K I D AAT CTC TAC TTC CAG GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC
R E N N Y F Q Q G
ACG TTA GGC ATC ATC CAG GAT AAC CTC ATC CTC ATC CTC ATC CTC ATC CTC ATC CTC ATC CTC ATC CTC
T L H V I I Q Q T
V H V V I I Q Q T
TAT GTC AAT TCT TCT TCT TCT TCT TCT TCT TCT TCT TCT TCT TCT TCT TCT TCT TCT TCT TCT TCT
Y V N S
CAG GAG CTC CTC GGC GAG CAG AGT GGA TAT CAC CAC GTG GAA CAG GAG AAA GAA GAA GAA GAA GAA
O E L
GGC AAC ACA GGG GAG CAG AGT GGA TAT CAC CAC GTG GAA CAG GAG AAA GAA GAA GAA GAA GAA GAA
G N T T G
GAA GAC CTC CCA GGC ACG GAA GAC TTT GTG GGC CAT CAA GGT ACT GTG GTG GTG GTG GTG GTG GTG
E D L L P P G G T
GAT TCA CAA GGG AGG AAT TGT AGT ACA AAT GAC TCA CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG

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Modifications to the μ opioid receptor to add TEV protease sites



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μ Opioid receptor DNA and Protein sequence

```
ATG GAC AGC AGC AGC GCT GCC CCC ACG AAC GCC AGC AAT TGC ACT GAT GCC TTG GCG TAC TCA AGT
M   D   S   S   S   A   A   W   T   N   A   S   V   N   C   T   T   A   L   A   G   A   G   S   S
TGC TCC CCA P   A   P   CCG AAC S   W   TCC TCC AAC AAC GGC GGC GGC GGC GGC GGC GGC GGC GGC
C   S   P   G   A   P   CCG AAC S   W   TCC TCC AAC AAC GGC GGC GGC GGC GGC GGC GGC GGC GGC
GAC CCA TGC TCC AGC ATC ACG ATC ATC ATC ATC ATC ATC ATC ATC ATC ATC ATC ATC ATC ATC ATC
D   P   C   G   G   M   N   T   T   T   T   T   T   T   T   T   T   T   T   T   T   T   T   T   T
AGT CCC TCC AGC ATC ACG ATC ACG ATC ACG ATC ACG ATC ACG ATC ACG ATC ACG ATC ACG ATC ACG
S   P   S   G   A   A   T   T   T   T   T   T   T   T   T   T   T   T   T   T   T   T   T   T   T
CTC TTC GGA AAC TTT CTC ATC TTT CTC ATC TTT CTC ATC TTT CTC ATC TTT CTC ATC TTT CTC ATC
L   F   N   N   F   L   N   T   T   T   T   T   T   T   T   T   T   T   T   T   T   T   T   T   T
N   N   N   N   N   N   N   N   N   N   N   N   N   N   N   N   N   N   N   N   N   N   N   N
AGT GTG AAT TAC ATT TAC ATT TAC ATT TAC ATT TAC ATT TAC ATT TAC ATT TAC ATT TAC ATT
S   V   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y
ATA GAT TAC TAT TAC TAT TAC TAT TAC TAT TAC TAT TAC TAT TAC TAT TAC TAT TAC TAT TAC
I   D   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y
ATT GCA TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC
I   A   V   C   N   N   N   N   N   N   N   N   N   N   N   N   N   N   N   N   N   N   N   N
AAT GTC TGC AAC TGC AAC TGC AAC TGC AAC TGC AAC TGC AAC TGC AAC TGC AAC TGC AAC
N   V   C   N   N   N   N   N   N   N   N   N   N   N   N   N   N   N   N   N   N   N   N   N
AAA TAC AGG CAA Q   K   L   K   L   K   L   K   L   K   L   K   L   K   L   K   L   K   L
AAC CTG CTG AAG CAA Q   K   L   K   L   K   L   K   L   K   L   K   L   K   L   K   L
TGC TAT GGA CTG AAG CAA Q   K   L   K   L   K   L   K   L   K   L   K   L   K   L
C   Y   G   L   L   L   L   L   L   L   L   L   L   L   L   L   L   L   L   L   L   L
GAC AGG AAT CTT CGA R   R   L   L   L   L   L   L   L   L   L   L   L   L   L   L
TGG ACT CCC ATT CAC R   R   L   L   L   L   L   L   L   L   L   L   L   L
W   T   P   I   H   H   H   H   H   H   H   H   H   H   H   H   H   H   H   H
CAG ACT GTT TCT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT
Q   T   V   S   W   W   W   W   W   W   W   W   W   W   W   W   W   W   W   W
CTT TAT GCA TTT F   D   D   D   D   D   D   D   D   D   D   D   D   D   D   D
L   Y   A   F   L   L   L   L   L   L   L   L   L   L   L   L   L   L   L   L
TCC AAC ATT GAG CAA Q   O   O   O   O   O   O   O   O   O   O   O   O   O   O
S   N   N   A   A   A   A   A   A   A   A   A   A   A   A   A   A   A   A   A   A
GCC AAT ACA GTG GAT D   D   D   D   D   D   D   D   D   D   D   D   D   D
```

FIG. 17

[illegible]

FIG. 18

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μ Opioid receptor with TEV site in 3rd intracellular loop

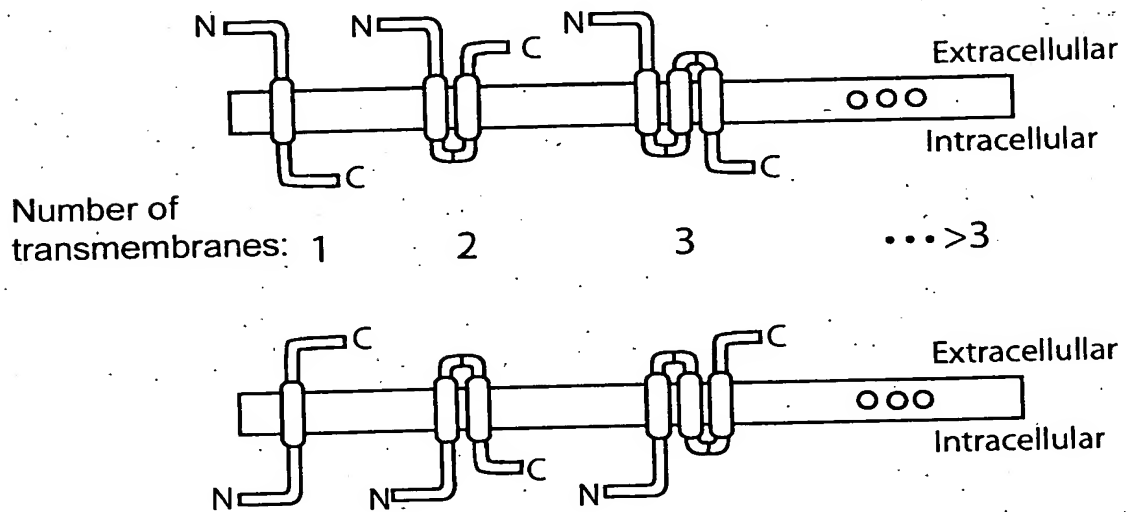
ATG GAC AGC AGC GCT GCC CCC ACG AAC GCC AGC AAT TGC ACT GAT GCC TTG GCG TAC TCA AGT
M D S S A GCA CCC AGC CCC GGT TCC TCC TGG TGC AAT TGC ACT GAT GCC TTA L L GAT GGC GAC CTG TCC
C S P A A P S S W CTG GGC GGC GGC AAT TGC TCC CAC H H GAC AGC CTG L L CTG TTA L L GAT GGC GAC
GAC CCA TGC GGT CCG AAC CGC ACC GAC GAC CTG GGC GGC GGC AAT TGC TCC TAC TGC TCC TCC TCC TCC TCC
D P C G G P N N R T T I T T I T T I T T I T T I T T I T T I T T I T T I T T I T T I T T I T T I T T I T
AGT CCC TCC ATG ATC ACG GCC GCT ATG ATG GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC
S P S M I T A I I T
CTC TTC GGA AAC TTC CTG GTC ATG ATG GTC ATT GTC AGC AAT TGC TCC TCC TCC TCC TCC TCC TCC TCC TCC
L F G N F L L V V L
AAC ATC TAC ATT TTC AAC AAC CTG GCA GAT GGC TTA ACC ACC ACC ACC ACC ACC ACC ACC ACC ACC ACC ACC
N I Y I T A T
AGT GTG AAT TAC CTA ATG GGA ACA TGG CCA TTT GGA ACC ATC ATC ATC ATC ATC ATC ATC ATC ATC ATC ATC
S V N Y Y L L M M G G L
ATA GAT TAC TAT AAC ATG AAC ATG TTC ACC ACC ACC ACC ACC ACC ACC ACC ACC ACC ACC ACC ACC ACC ACC
I D Y Y C
I A V C N N W TGG ATC CTC TCT TCA GAT TCA GAT TCA GAT TCA GAT TCA GAT TCA GAT TCA GAT TCA GAT TCA
AAT GTC TGC AAC TGC AAC TGC AAC TGC AAC TGC AAC TGC AAC TGC AAC TGC AAC TGC AAC TGC AAC TGC AAC
N V C N N W TGG ATC CTC TCT TCA GAT TCA GAT TCA GAT TCA GAT TCA GAT TCA GAT TCA GAT TCA GAT TCA
AAA TAC AGG CAA G GGT TCC ATC CTC TCT TCA GAT TCA GAT TCA GAT TCA GAT TCA GAT TCA GAT TCA GAT TCA
K Y R Q L
AAC CTG CTG AAG ATC
N L
TGC TAT GGA AAG ATC
C Y G L
GAC GAA AAC CTC TAC TTC CAG GGC AGG AAT CTT CGA AGG ATC ATC ATC ATC ATC ATC ATC ATC ATC ATC ATC
D E N V F I ACG TTC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC
GTG GCT GTG TTC ATC
V A V CCA GAA ACT
ACA ATC CCA GAA ACT
T I I P E L
AAC AGC TGC CTC AAC CCA GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC
N S C L N P V
GAG TTC TGT ATC CCA ACC TCT TCC AAC ATT GAG CAA CAA CAA CAA CAA CAA CAA CAA CAA CAA CAA CAA CAA
E F C I I P T S
ACT AGA GAC CAC CCC TCC ACG GCC AAT ACA GTG GAT AGA ACT AAT AAT AAT AAT AAT AAT AAT AAT AAT AAT
T R D H P S T A N T V D R T N H Q R S L C

FIG. 19

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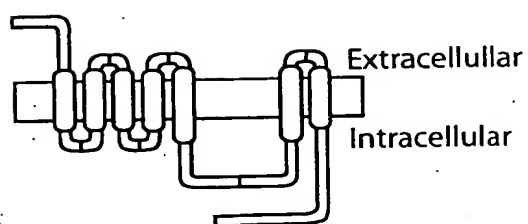
FIG. 20

Generic MSST Structure

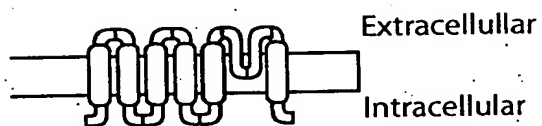


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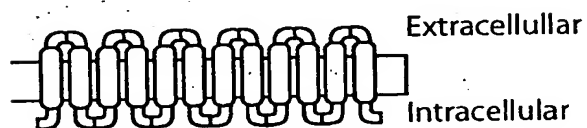
FIG. 21



GPCR



Potassium Channel



Transporter